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ЗА ПРИРОДНЕ НАУКЕ

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## **GIBBERELLA FUJIKUROI (SAWADA) WOLLENWEBER, THE NEW PARASITICAL FUNGUS ON RICE IN THE REPUBLIC OF MACEDONIA**

**ABSTRACT:** The recent observation of *Gibberella fujikuroi* (Sawada) Wollenworth (teleomorph) (anamorf: *Fusarium moniliforme* Sheld.) *Fusarium fujikuroi* Nirenberg (anamorph), *F. moniliforme* J. Sheld. (synonym), the causal agent of Bakanae disease in rice fields, provides an opportunity to observe the characteristics of the pathogen and the possibility of prevention in Kocani area. Plant material with *Gibberella fujikuroi* symptoms was collected from rice (*Oryza sativa*) over the period of 3 years (from 2006 to 2008). Within this study, the presence and damage caused by this pathogenic fungus were confirmed. The objective of this study was a continuous field observation of symptoms in order to establish the percentage of infection and use of laboratory methods for proper protection.

**KEY WORDS:** Bakanae, *Gibberella fujikuroi*, Kocani, rice fields

### **INTRODUCTION**

Rice (*Oryza sativa* L.) is an important crop in the Republic of Macedonia, and is cultivated in over 2991,55 ha, from which 2840,60 ha are located in the area of Kočani (National Statistics of Macedonia, 2007). Among various diseases, which effect the crop production, Bakanae is the leading one.

The name Bakanae means "bad" or "naughty" seedlings in Japanese, referring to the elongation symptoms specific for the disease, and caused by gibberellin production of the pathogen upon infection of the host. The causal agent of Bakanae disease of rice is fungus (*Gibberella fujikuroi*, Sawada, Wollenworth — teleomorph, (anamorf: *Fusarium moniliforme* Sheld.), *Fusarium fujikuroi* Nirenberg (anamorph), *F. moniliforme* J. Sheld. synonym). The fungus produces gibberellins and other secondary metabolites such as carotenoids, bikaverin and fusarin, which directly affect the rice growth.

Bakanae is a monocyclic disease and the pathogen is often spread predominantly with infected seeds, although infected crop residue from the previous season may also serve as a source of inoculum.

Bakanae is traditionally associated with rice, but water grass plants such as *Echinochloa* spp., with classic symptoms of Bakanae were also observed in California in 2002 (Carter, L. A., 2008). Although the Bakanae disease usually causes dieback or sterility of rice, mycotoxin contamination also presents a concern since the pathogen is seed-borne.

The teleomorph, *Gibberella fujikuroi*, has been reported on rice in China, Japan and Taiwan, (Sun, 1975; Sung and Snyder, 1977; Watanabe and Umehara, 1977). Ascospores are reported as the primary source of inoculum for Bakanae in Taiwan (Sun, 1975).

### *Disease Symptoms*

Symptoms vary depending on the strain, inoculum levels and the presence of toxin. High inoculum density leads to seedling blight, stunting and chlorosis. Low inoculum levels result in etiolation symptoms due to gibberellin production.

Typical symptoms caused by *Gibberella fujikuroi* are usually characterized with:

- infected plants that are several inches taller than normal plants in seedbed and field;
- thin plants with yellowish green leaves and pale green flag leaves;
- dieback seedlings at early stage;
- reduced and dried of leaves at late infection;
- partially filled sterile or empty grains on surviving plant at maturity;
- infected seedlings with lesions on roots, that die before or after.

Moreover, there is no other disease with symptoms similar the those of the Bakanae disease of rice.

### *Characteristics of Gibberella fujikuroi*

The pathogen sexually produces ascospores that are formed within a sac known as ascus. Asci are contained in the fruiting bodies called ascocarps which are referred to as perithecia. The perithecia are dark blue and measure 250—330 x 220—280  $\mu\text{m}$ . They are spherical to oval and somewhat roughened outside. The asci are cylindrical, piston-shaped, flattened above, and their dimensions are 90—102 x 7—9  $\mu\text{m}$ . They are 4, 6 but seldom 8-spored. The spores are one-septate and about 15 x 5.2  $\mu\text{m}$ . They are occasionally larger, measuring 27—45 x 6—7  $\mu\text{m}$ .

The anamorph produces gibberellin and fusaric acid. Biological studies of the two substances showed that fusaric acid causes stunting and gibberellin causes elongation.

Hyphae are branched and septated. The fungus has micro and macroconidiophores bearing micro and macroconidia, respectively. The microconidiophores are single, lateral, and subulate phialides. They are formed from aerial hyphae. The microconidia are, more or less, agglutinated in chains and remain

joined or cut off in false heads. They are later scattered in clear yellowish to rosy white aerial mycelia as a dull, colorless powder. They are 1—2 celled and fusiform oval.

The macroconidiophores have basal cells with 2—3 apical phialides, which produce macroconidia. The macroconidia are delicate, slightly sickle-shaped or almost straight. They narrow at both ends and are occasionally somewhat bent into a hook at the apex and distinctly or slightly foot-celled at the base.

The sclerotia are 80 x 100  $\mu\text{m}$ . They are dark blue and spherical. The stroma are more or less plectenchymatous and yellowish, brownish, or violet.

## MATERIAL AND METHODS

### *Collection of samples*

### *Field analyses of plant material*

Plant material with symptoms similar to that caused by *Gibberella fujikuroi*, was collected from rice (*Oryza sativa*) during the period of 3 years (2006—2008). Each year, collections were made throughout the whole rice growing season. The seedlings with early symptoms of Bakanae disease, particularly chlorosis and elongation, were collected from rice fields in Kočani areas. Later in the season, plants showed visible symptoms in the field (Figure 1). During this period, 10 different fields were observed, and 5 infected sam-



Fig. 1 — Visible symptoms in the field caused by *Gibberella fujikuroi*



Fig. 2 — Abnormal oblong stem and leaves

ples, were collected. Symptomatic plants have thin stem, 30 cm higher than normal, and leaves are usually yellowish green and elongated in comparison with normal leaves (Figure 2).

#### *Isolation of the pathogen*

Isolation of the pathogen was made from the stem and leaves of the symptomatic plants collected from the rice fields in Kočani areas. They were cut into 1 cm<sup>2</sup> pieces, sterilized with 0.1% aqueous solution of mercuric chloride, rinsed twice with distilled water and then placed on potato dextrose agar medium (PDA). The plates were incubated at  $27 \pm 2^{\circ}\text{C}$  for 5 to 7 days. After the development, isolated fungi were identified according to Nelson et al. (1983).

#### *Pathogenicity testing*

All collected isolates were screened for ability to induce symptoms of Bakanae on rice seedlings. Isolates grew on the potato dextrose agar for 10 to 15 days, until the mycelia clearly emerged. When mycelia fullfilled Petri dishes, one small piece of mycelia with agar was cut and used to make a suspension with sterile distilled water. Mycelium was scraped with a sterile glass slide. Ten seeds of different rice cultivars (San Andrea and Monticelli), were soaked in 25 ml of suspension for 1 h at  $25^{\circ}\text{C}$ . The control seeds were soaked only in sterile distilled water (Figure 3). The experiment was conducted during May in

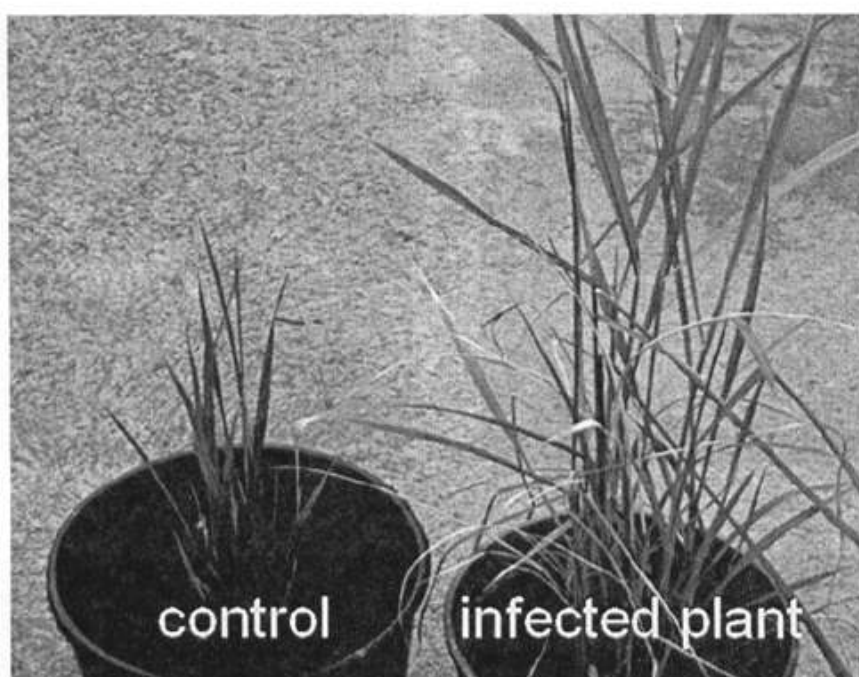


Fig. 3 — Test of pathogenicity — control with sterile water and infected plant with suspension from *Gibberella fujikuroi*

an open environment and data for Bakanae symptoms, expressed in percentage of plant infection were evaluated 60 days after sowing.

## RESULTS

### *Morphology*

After field analysis, a laboratory testing for detection and identification of rice pathogen was conducted. Thirty morphologically consistent isolates gained from the plant material with typical Bakanae symptoms were analysed. All isolates on potato dextrose agar (PDA), established the same mycelia. Clear culture had mycelia with white color on the top of Petri dishes and the mycelia was redish to brown coloured on the underside. In our case, all Petri dishes were with lilac colour on the underside (Figure 4).

The fungus has micro and macro conidiophores bearing micro and macro conidia, respectively (Figure 5). Microconidiophores are single, lateral and formed from hyphae, while macroconidiophores consist of a basal cell bearing 2—3 apical phialides which produce macroconidia. Macroconidia are multi-celled (3 to 7 septate), slightly curved or bent at pointed ends, typically canoe-shaped and measure 25—60 x 2.5—4  $\mu\text{m}$ . Microconidia are one-celled, ovoid or oblong, borne singly in chains or false head on laterally borne conidiophores and measure 5—12 x 1.5—2.5  $\mu\text{m}$ . Some conidia are intermediate, with two or three cells, oblong or slightly curved.



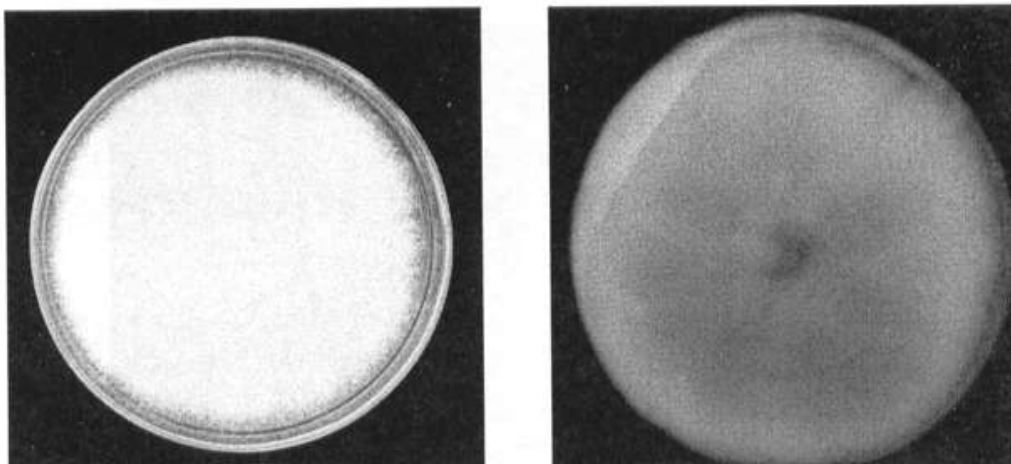


Fig. 4 — Mycelia from parasitic fungus *Gibberella fujikuroi*

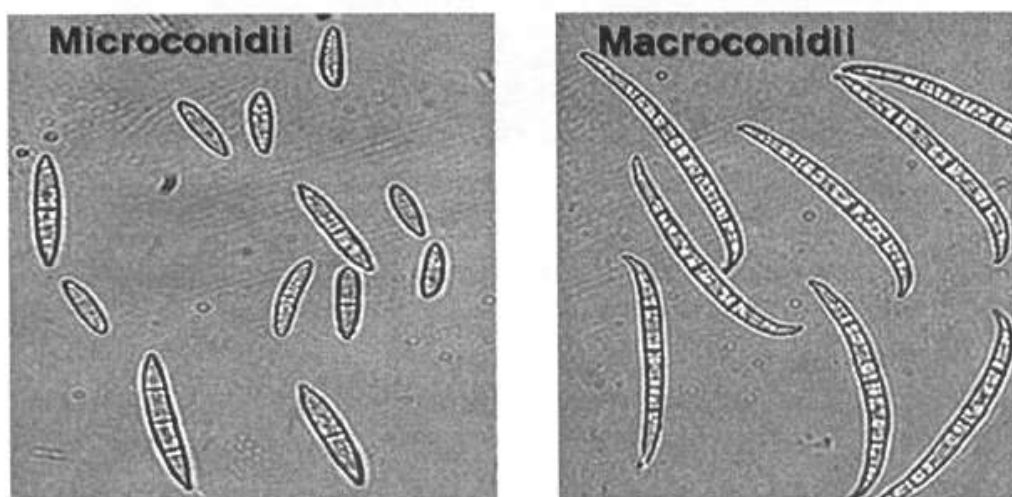


Fig. 5 — Micro and macroconidia from *Gibberella fujikuroi*

The results from pathogenicity testing showed visible symptoms on inoculated plants. The ten seeds of different rice cultivars (San Andrea and Monticelli), which were soaked in 25 ml of suspension for 1 h at 25°C, after 60 days showed visible symptoms in comparison with the control seeds, which were soaked only in sterile distilled water.

Symptomatic plants have stem 30 cm higher than normal, and leaves are usually chlorotic and elongated when compared to the normal ones, with angle position of 90°C.

## DISCUSSION

The teleomorph, *Gibberella fujikuroi*, has been reported on rice in China, Japan and Taiwan (Sun, 1975; Sung and Snyder 1977; Watanabe



and Umehara 1977). Ascospores are reported as the primary source of inoculum for Bakanae in Taiwan (Sun, 1975).

Bakanae is a serious disease endangering rice fields in the Republic of Macedonia, and with this observation, the presence and damage caused by this pathogenic fungus were confirmed.

The seeds are usually infected during the flowering stage of the crop. Severely infected seeds are discolored because of the pathogen conidia. Seed infections occur via airborne ascospores and lead to conidia that contaminate the seed during harvesting. Discoloured seeds give rise to stunted seedlings, whereas the infected seeds without discolouration produce seedlings with typical Bakanae symptoms. Infections may also take place with spores and mycelium, which are left in the water used for seed soaking.

The fungus infects plants through roots or crowns. It later becomes systemic, i.e. it grows within the plant and systemically infect the panicle. The microconidia and mycelium of the pathogen are found to be concentrated in the vascular bundles, particularly large pitted and xylem vessels.

Sine the production of gibberilin has not been investigated in Macedonia, our future observations are planned to focus on gibberellin production by the pathogen upon infection of the host.

### *Control and prevention*

Clean seeds should be used to minimize the occurrence of the disease. Salt water can be used to separate lightweight and thereby reduce seedborne inoculum. In our observation, we did not treat our seeds, but the already published studies proved that seed treatments using fungicides such as thiram, thiophanate-methyl, or benomyl are effective before planting. Benomyl or benomyl-t applied at dose of 1–2% (seed weight basis) should be used for dry seed coating. However, rapid increase of resistance against benomyl and carbendazim has been reported which may be caused by their successive applications. Triflumizole, propiconazole and prochloraz were found to be effective against strains that are resistant to benomyl and the combination of thiram and benomyl.

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**GIBBERELLA FUJIKUROI (SAWADA) WOLLENWEBER, НОВА ПАРАЗИТНА  
ГЉИВА НА ПИРИНЧУ У РЕПУБЛИЦИ МАКЕДОНИЈИ**

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**Резиме**

У ова истраживања били су укључени узрочници Бакане болести на пиринчаним пољима око Кочана, са циљем да се испитају карактеристике патогена *Gibberella fujikuroi*, као и могућност заштите пиринча. У трогодишњем периоду, од 2006. до 2008. године, биљни материјал са симптомима *Gibberella fujikuroi* био је прегледан и колекциониран са пиринча (*Oryza sativa*). Овим детаљним испитивањима потврђени су присуство и штета прузрокована овом патогеном гљивом. Циљ овог истраживања је био да се континуирано прате симптоми болести са поља да се утврди проценат инфекције, а такође преко лабораторијских метода омогући одговарајућа заштита.